Deficiency for mannan-binding lectin is associated with antibodies to *Saccharomyces cerevisiae* in patients with Crohn's disease and their relatives

Mannan-binding lectin (MBL) is a member of the collectin family and is an important component of the innate immune response. Three different MBL alleles in coding exon 1 (point mutations in codons 52, 54 and 57) result in low serum levels of MBL.[1] Earlier, we described cellular and humoral immune reactivity of a subgroup of patients with Crohn's disease to mannans, a cell wall antigen of yeasts.[2] The humoral immune response in these patients is commonly known as antibodies to *Saccharomyces cerevisiae* (ASCA). In a previous paper, we found considerably more patients with Crohn's disease with low MBL serum levels to be ASCA positive than in the subgroup with high MBL levels. The homozygotic or compound heterozygotic mutations in the exon were associated with the development of ASCA in patients with Crohn's disease.[3]

This is in contrast with the data reported in this journal by another group that found an increased prevalence of ASCA in patients with MBL mutations in exon 1, which however, did not reach significance in their large independent cohort of patients with Crohn's disease.[4] In this paper, we confirmed in another cohort that patients with low serum MBL or MBL deficiency were considerably more often ASCA positive as compared with patients with normal levels of MBL. Low MBL levels (defined as <500 ng/ml) were found in 30 of 52 (58%) patients with Crohn's disease; 26 (87%) of these patients were ASCA positive. By contrast, only 9 (41%) patients with normal MBL levels were ASCA positive ($p<0.001$, Fisher's, exact test; fig 1). There are several possible explanations for the observed differences found in comparison to the above-mentioned report[4]: (1) the method of the ASCA test that was used; (2) the genetic difference due to the geographically different origins of the populations; and (3) a bias towards a population of patients with Crohn's disease with a more severe disease course seen at the University Hospital of Bern, Switzerland. To extend our study and to evaluate the relationship between MBL deficiency and generation of ASCA, the second part of our study focused on the ASCA and the MBL status of the healthy family members of patients with Crohn's disease. A total of 158 family members of 53 patients agreed to provide clinical data and blood samples. ASCA were found in 38 of 158 (24%) family members. A similar prevalence was observed in previously published papers.[5–7] All 46 (29%) family members had low MBL levels (<500 ng/ml). This population was analysed for its ASCA status; 23 (50%) family members were ASCA positive and 23 were ASCA negative (fig 1). This was in contrast with 112 family members with normal MBL levels; among those, 15 (13%) were ASCA positive and 97 were ASCA negative ($p<0.001$). In 16 family members, MBL mutations leading to MBL deficiency were found (B/B, four relatives; D/D, three relatives; and nine compound heterozygotic people). Further, seven patients were genotyped as either LXPA/LYPD or LXPA/LYPB and had a MBL concentration <100 ng/ml. Relatives with mutations leading to MBL deficiency had a significantly greater prevalence of ASCA compared with the 135 relatives with normal MBL values ($p = 0.018$, χ^2 test). Thus, our analyses clearly show an association of ASCA positivity with MBL deficiency in patients with Crohn's disease and also in their healthy family members.

Therefore, our paper provides further evidence that genetically altered MBL levels in patients with Crohn's disease and their relatives could be, at least partly, responsible for the enhanced immune reactivity to yeast antigens seen in a subgroup of these patients and their relatives. However, other factors also contribute to the development of this unusual immune reaction, as there are MBL-deficient healthy people who are ASCA negative.

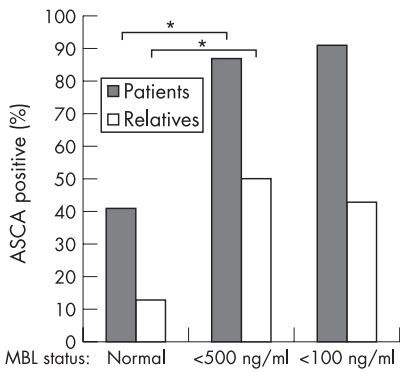

Figure 1 Incidence of antibodies to *Saccharomyces cerevisiae* (ASCA) in patients (black) and in healthy relatives (white) depends on the serum mannan-binding lectin (MBL) level. ASCA and MBL levels were measured by ELISA. ASCA was significantly more prevalent in people with low MBL values ($p<0.001$ for patients and $p<0.001$ for relatives) than in people with normal MBL levels.

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Molecular comparison of dominant microbiota associated with injured versus healthy mucosa in ulcerative colitis

Ulcerative colitis is an inflammatory bowel disease (IBD) characterised by a chronic inflammation of the colorectal mucosa. The role of the gut microbiota in triggering or maintenance of the mucosal inflammation is strongly suspected.[1] We may postulate that a localised dysbiosis could explain the localisation of the lesions. Many of the recent studies have been made possible by the development of culture-independent techniques, which identify bacteria on the basis of comparisons of the nucleic acid sequence of rRNA molecules. Temporal temperature-gradient gel electrophoresis (TTGE) showed that in healthy people dominant microbiota associated with colonic mucosa is similar from the caecum to the rectum.[2] This has also been observed in Crohn's disease,[2] and the dominant microbiota associated with ulcerated mucosa did not differ markedly from the microbiota associated with non-ulcerated mucosa.[3]

The aim of this study was to compare the dominant microbiota associated with injured colonic mucosa versus healthy colonic mucosa, using TTGE in patients with active ulcerative colitis.

Ten patients with active ulcerative colitis were studied, after informed consent was obtained. Three patients had a proctitis and seven had a left-sided colitis. None of them had received antibiotics or sulfasalazine within the previous 3 months. Four patients were receiving corticosteroids, four mesalazine and three azathioprine. Colonoscopy was performed during ulcerative colitis flare-ups. Colonic or rectal biopsy samples were collected from both injured and healthy mucosa in each patient

and were frozen immediately in liquid nitrogen, and stored at −80°C until analysis.

Global analysis of TTGE profiles showed a high biodiversity in both injured colonic mucosa and healthy colonic mucosa. Mean (standard deviation (SD)) numbers of bands were 11.5 (2.7) and 11.6 (2.6) in injured colonic mucosa and healthy colonic mucosa microbiota, respectively (no marked differences). Figure 1 shows the TTGE profiles of representative patients.

Intraindividual analysis showed that in a given patient, the mean (SD) similarity index between injured colonic mucosa and healthy colonic mucosa TTGE profiles was 94.9 (4.6%). Similarity indexes of TTGE profiles of samples collected from a given segment in different patients ranged from 59.5 (15.4%) to 79.2 (8.2%) for injured colonic mucosa and from 62.4 (16.3%) to 71.2 (13.4%) for healthy colonic mucosa (table 1). The mucosa-associated microbiota differed markedly between patients. However, these differences were comparable in injured and healthy mucosa and were within the range of inter-individual variability. The interindividual similarity index of each healthy colonic mucosa or injured colonic mucosa segment was always significantly lower than the intraindividual similarity index between injured colonic mucosa and healthy colonic mucosa (table 1).

All TTGE profiles were compared in a single dendrogram. The branching distances between two samples shows their degree of relatedness. Except for patient 3, samples from the same patient clustered together (fig 2).

The role of the intestinal microbiota in the pathogenesis of ulcerative colitis is suspected.[1] This could involve a dysbiosis or an altered immune response to a normal microbial context.[1] We showed, using TTGE, that the dominant mucosa-associated microbiota in patients with ulcerative colitis does not differ between the injured and the healthy mucosa.

A previous study using the same method also showed no sign of localised dysbiosis in the dominant mucosal microbiota during Crohn's disease.[3] However, these two studies cannot exclude quantitative differences in the microbiota or qualitative differences in the subdominant bacteria. Kleessen *et al*[4] found more bacteria on the mucosal surface of patients with IBD than on those of controls without IBD. A decrease in the biodiversity of the active portion of the fecal microbiota has been reported in ulcerative colitis.[5] Restricted biodiversity has also been observed in mucosa-associated microbiota by Ott *et al*.[6] However, in this study the dominant microbiota biodiversity, assessed by the number of bands on the TTGE, was similar in healthy colonic mucosa and in injured colonic mucosa. These results suggest that the dominant mucosa-associated microbiota does not differ in ulcerated tissues during IBD; further studies searching for a hypothetical localised dysbiosis to explain the patchy distribution of the disease should thus focus on other targets, and probably to our view on usually subdominant bacteria such as *Escherichia coli* or sulphate reducing bacteria (which have been suspected to play a part in IBD in other studies).[5 7]

Table 1 Comparison of interindividual similarity index of each healthy colonic mucosa or injured colonic mucosa segment versus mean intraindividual similarity index between injured colonic mucosa and healthy colonic mucosa.

	Healthy mucosa		Injured mucosa	
	Right colon	Left colon	Left colon	Rectum
Number of samples	6	4	7	3
Mean (SD) similarity index (%)	62.4 (16.3)	71.2 (13.4)	59.5 (15.4)	79.2 (8.2)
Versus mean intra-individual similarity (p value)	0.001	0.004	0.001	0.001

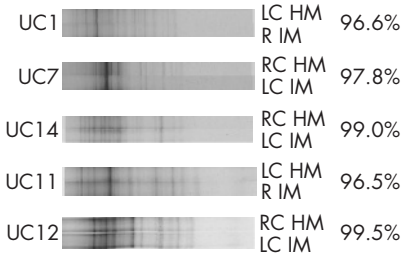

Figure 1 Temporal temperature-gradient gel electrophoresis of 16S rDNA amplicons (obtained using primers for the V6–V8 regions) extracted from biopsy samples of healthy and injured mucosa from the colon or the rectum of five patients with ulcerative colitis (UC). HM, healthy mucosa; IM, injured mucosa; LC, left colon; R, rectum; RC, right colon; Right side, similarity indexes (expressed in %) paired samples.

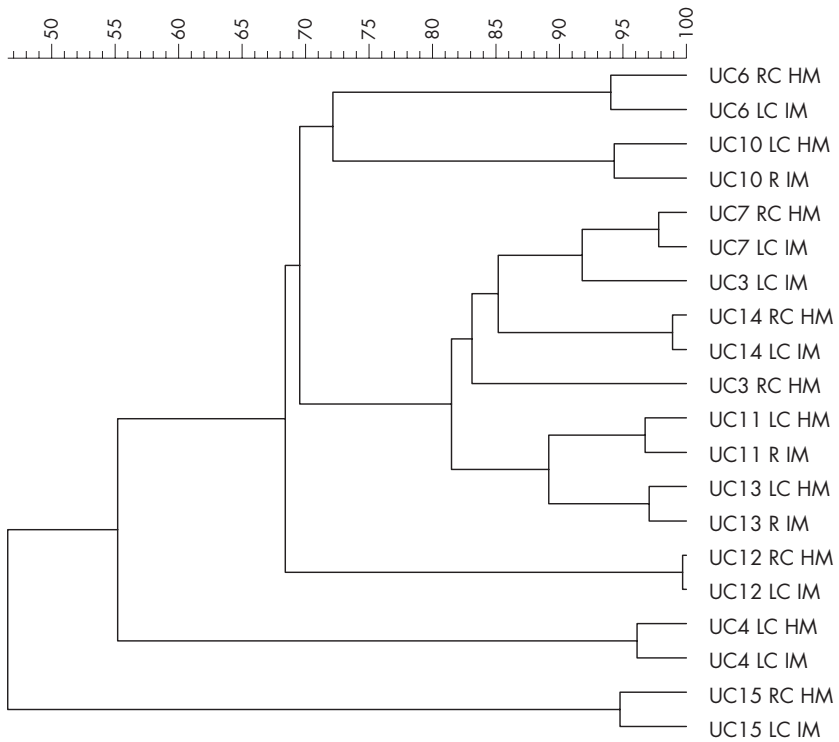

Figure 2 Dendrogram representing the temporal temperature-gradient gel electrophoresis (TTGE) profiles of 16S rDNA amplicons (obtained using primers for the V6–V8 regions) extracted from biopsy samples of injured mucosa (IM) and healthy mucosa (HM) of 10 patients with ulcerative colitis (UC). The dendrogram represents a statistically optimal representation of the similarities between TTGE profiles on the basis of the matrix of Pearson's correlation coefficients and applying the unweighted pair group method using arithmetic averages. LC, left colon; R, rectum; RC, right colon.

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Limitations of non-ceruloplasmin-bound copper in routine clinical practice

We read with interest the paper on Wilson's disease by Merle *et al* (this issue, p 115). Some aspects of the investigation profile are omitted from the paper. Although data on the prevalence of reduced ceruloplasmin levels and raised non-ceruloplasmin-bound copper levels are provided outside cut-offs, data on mean (and median) levels, SD (interquartile ranges) and any skew or kurtosis are not. This would be useful as the pattern of result distributions in patients with Wilson's disease has substantial implications for the validity of diagnostic algorithms. In addition, this cohort survey clearly showed that some patients with Wilson's disease have ceruloplasmin concentrations within the reference interval (11.8%)—an important message when devising a diagnostic algorithm for Wilson's disease.

According to Roberts and Schilsky,[1] the upper end of the reference interval for non-ceruloplasmin-bound serum copper (NCC) is 15 μg/dl (2.4 μmol/l) and, in most patients with untreated Wilson's disease, the concentration is >25 μg/dl (3.9 μmol/l). They correctly went on to state that interpretation is difficult as the NCC depends on satisfactory copper and ceruloplasmin assays. Since then, we have shown that the upper reference interval in our population was 40 μg/dl (6.3 μmol/l)[2] but that the lower reference interval was −18.4 μg/dl (−2.9 μmol/l), which is clearly not possible and thus supports the assertion by Roberts and Schilsky about the need for satisfactory copper and ceruloplasmin assays. Furthermore, 40 μg/dl is considerably higher than the cut-off of 25 μg/dl used by Merle *et al* to detect 86.6% of patients with Wilson's disease; accordingly fewer patients would be detected using a more appropriate NCC cut-off. This, with the negative values found in 20% of normal patients, means that the NCC is a poor diagnostic test for the detection of Wilson's disease.

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Heredity and DNA methylation in colorectal cancer

In their interesting review of colorectal cancer (CRC) as a model for epigenetic tumourigenesis, Wong, *et al* (in this issue, p 139) discuss the role of hereditary factors in explaining the aetiology of CRC with DNA methylation. Firstly, they assert that larger studies do not support a hereditary aetiology for the CpG island methylator phenotype (CIMP). Secondly, they suggest that *MLH1* epimutation (or germline hemi-allelic methylation) may be heritable. Both of these propositions may be challenged.

In the case of an inherited predisposition to CRCs with acquired DNA methylation or CIMP, a family cancer clinic-based study that excluded families with the Lynch syndrome, found that members with CRCs showing the CIMP had a 13-fold increased risk of having a first-degree relative with cancer (not necessarily CRC) as compared with those without CIMP-positive CRC.[1] A hospital-based study by Ward *et al*[2] could not confirm this finding, but it is pertinent that they excluded families considered to have hereditary nonpolyposis colorectal cancer (HNPCC). Ward *et al* regularly use clinical definitions for HNPCC.[3] The exclusion of ''HNPCC'' families is likely to have introduced a major bias, as not all families meeting a clinical definition for HNPCC (eg, the Amsterdam criteria) in fact show evidence of DNA mismatch repair deficiency that would be indicative of a germline defect in a DNA mismatch repair gene.[4]

The second large study cited by Wong *et al*[5] was a population-based series that assessed CIMP in 864 CRCs and defined CIMP-high as the presence of methylation in at least two of five markers.[5] This is not a stringent definition of CIMP-high as indicated by the low frequency of *BRAF* mutations (32/182, 17.6%) among CIMP-high/DNA microsatellite stable (MSS) CRCs. As Wong *et al* point out, an inherent difficulty in establishing whether genetic factors may explain CIMP is the lack of an agreed definition of CIMP. It is clear, however, that mutation of *BRAF* cosegregates with extensive CIMP[6] and may therefore be used as a surrogate for high-level CIMP. In the same large population-based study group, but now stratified on the basis of *BRAF* mutation,[7] the findings were different. In the subset of MSS CRCs, the odds ratio for having a positive family for subjects with *BRAF* mutation-positive CRCs was 4.23 (95% confidence interval 1.65 to 10.84) (as compared with subjects with *BRAF* mutation-negative CRCs). However, among subjects with high microsatellite instability (MSI-H) CRCs, a stronger family history of CRC was observed when cancers did not have the *BRAF* mutation.[7] This is only to be expected, as subjects with MSI-H CRCs that lacked *BRAF* mutations were relatively young and a proportion would have Lynch syndrome.

In 2005, a further paper described a series of 11 Lynch syndrome-like families, in which some CRCs were MSI-H but others had low-level MSI or were MSS.[8] Both CRCs and polyps in these ''MSI-variable'' families showed frequent mutation of the oncogene *BRAF* or methylation of the CIMP marker MINT31.[8] Affected subjects also had serrated polyps and two had hyperplastic polyposis. On the basis of these observations, it was suggested that an inherited predisposition to acquired DNA methylation in somatic tissues could give rise to a ''serrated pathway syndrome''.[8] Should *MLH1* be implicated, then one might observe the development of CRCs that were MSI-H. This would only apply to a subset of CRCs, but could by chance, as in the case of a similar serrated pathway syndrome family described in 1997,[9] affect all CRCs tested in a single family.

Loss of imprinting (LOI) of *IGF2* in normal colonic mucosa and normal leucocytes has been associated with a personal and family history of CRC.[10] A study from Japan has shown that IGF2 LOI was more frequent in CRCs with the features of CIMP.[11] The link between CIMP and LOI may be explained by methylation of the H19 differential methylated region.[12] These observations provide further evidence for the heritability of CIMP.

Turning to germline hemi-allelic methylation of *MLH1* (or *MLH1* epimutation), Gazzoli *et al*[13] were the first to identify an early-onset CRC, in which one *MLH1* allele showed methylation. Interestingly, the same allele was also found to be methylated in the subject's lymphocytes. By elegantly exploiting the existence of a common polymorphism in the promoter region of *MLH1*, Gazzoli *et al* succeeded in showing that the wild-type *MLH1* allele had been lost in the CRC. On this basis, they introduced the concept of germline hemi-allelic methylation of *MLH1* as a cause of ''HNPCC''.[13] However, they were unconvinced that a methylated allele could be transmitted vertically from parent to child, and concluded that the finding was likely to be both rare and sporadic. Miyakura *et al*[14] reported four more examples of early-onset MSI-H CRC associated with germline hemi-allelic methylation of *MLH1*. Although the patients were ascertained through cancer family clinics, they did not have family histories suggestive of Lynch syndrome. They were merely young and some had multiple tumours consistent with Lynch syndrome. Again, Miyakura *et al*[14] did not